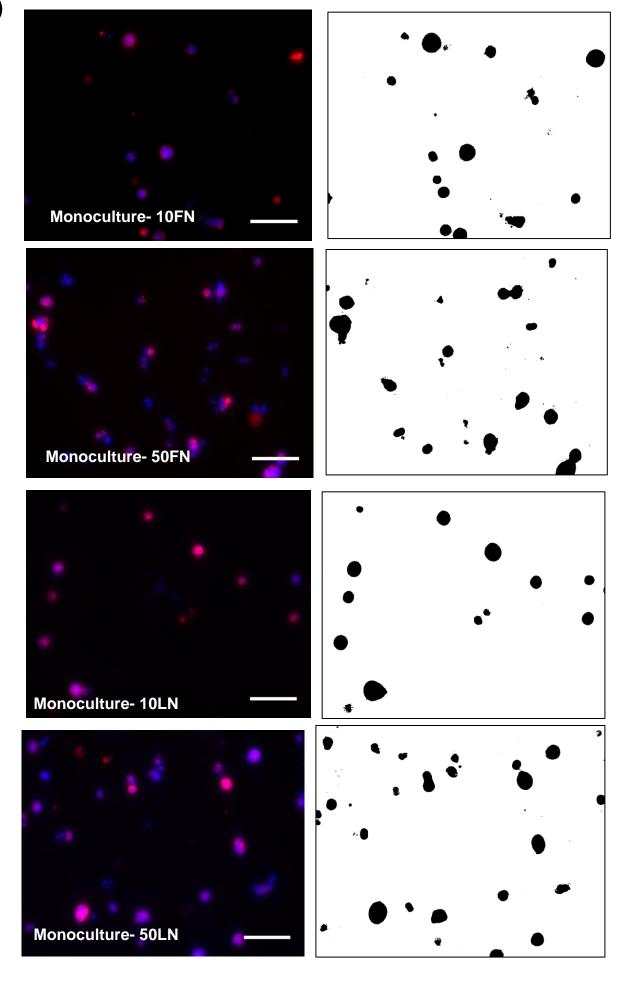
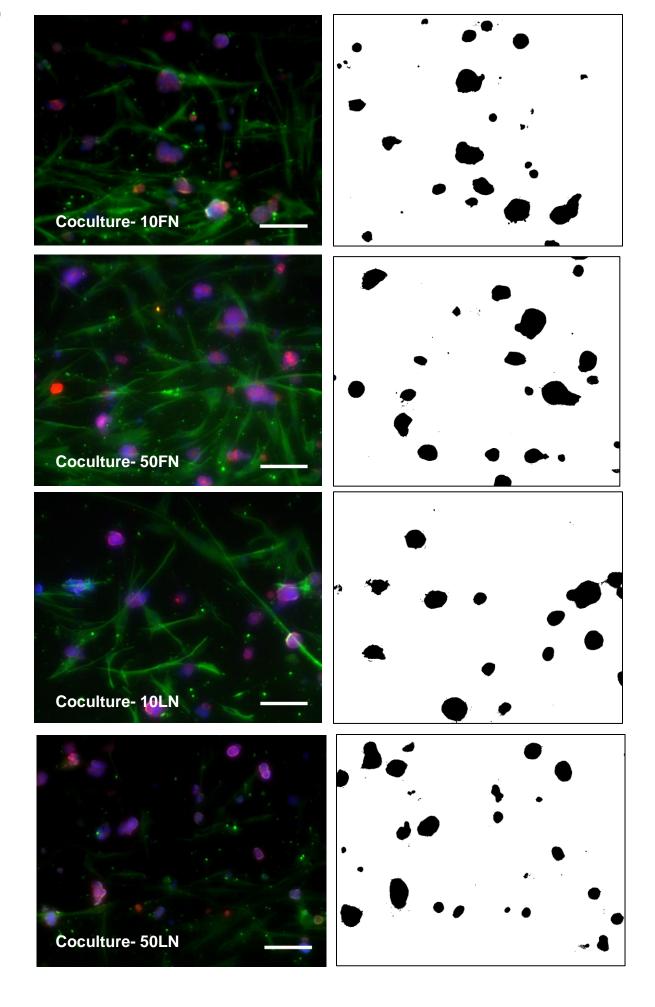
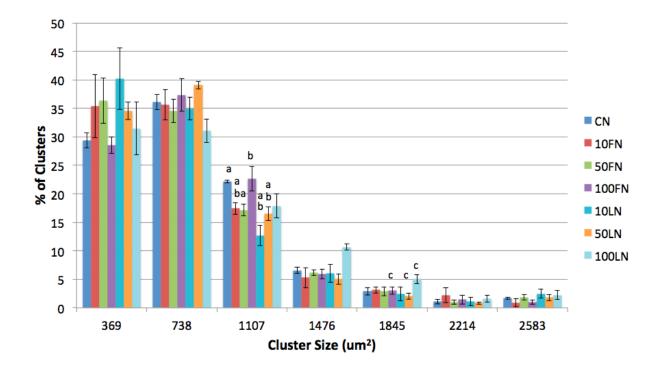
S1)



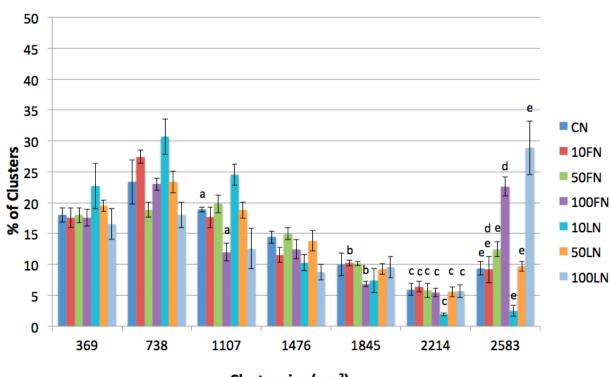
S2)







S4)



Cluster size (um²)



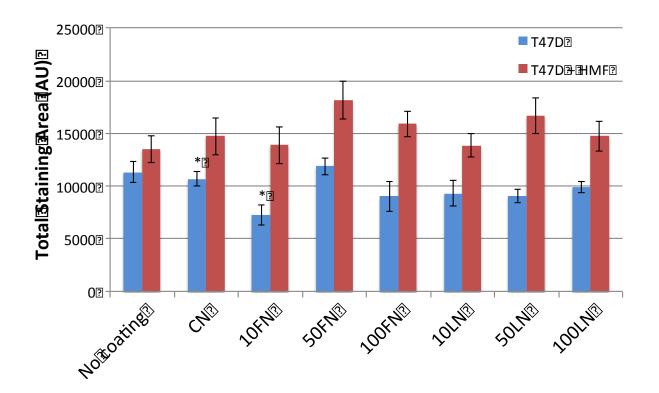


Figure S1- Immunofluorescence images (left panels) of T47D cells in monocultures inside microchannels for 7 days, fixed, and stained against cytokeratin (T47D cells, red), and Hoechst (nuclei, blue). Right panels show binary images of T47D clusters. Scale bars represent 100μm.

Figure S2- Immunofluorescence images (left panels) of T47D cells (red) co-cultured with HMF cells (green) inside microchannels for 7 days. Samples were fixed, and stained against cytokeratin (T47D cells), vimentin (HMF cells), and Hoechst (nuclei, blue). Right panels show binary images of T47D clusters. Scale bars represent 100µm.

Figure S3- Percentage of T47D clusters in monocultures with sizes varying from $369\mu m^2$ to $2583\mu m^2$. Each bar represents averages calculated from at least 4 replicates, and 2 separate experiments. p<0.05 within groups "a" thru "c", where group "a" was compared to CN, and group "b" to 100FN.

Figure S4- Percentage of T47D clusters in co-cultures with HMF cells with sizes varying from $369\mu\text{m}^2$ to $2583\mu\text{m}^2$. Each bar represents averages calculated from at least 4 replicates, and 2 separate experiments. p<0.05 in groups "a" thru "e", where group "c" was compared to 10LN, and group "e" to 100LN.

Figure S5- The proliferation of T47D cells on 2D ECM-coated microchannels was analyzed after 7 days of culture in monocultures (blue bars) and co-cultures with HMF cells (red bars) in different ECM compositions. Total staining area represents the total cell number of T47D cells inside microchannels. Averages were calculated from at least 4 replicates, and 2 separate

experiments. No significant differences were found between ECM compositions, with the exception of monocultures in CN and 10FN, n=4, *p<0.05